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RESEARCH IN PHOTOSYNTHESIS

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Photo-reactivation of Photosynthetic Reactions in Bean Leaves

Investigations on photo-restoration of the oxygen evolving system (Photosystem II) of dark, cold, aged bean leaves has been continued. Thus far our efforts have been concentrated towards restoration of Hill activity in inactive chloroplasts by addition of cofactors, compounds with oxidation-reduction capacity and protein-fractions from active chloroplasts from bean leaves in an attempt to localize the lesion in photosystem II. Hopefully such studies may give information concerning some of the compounds of photosystem II.

Thus far we consistently have observed "reactivation" only with α-Tocopherol ($E^{1}_{o} = 0.35$). Results are complicated by a light sensitized reduction of ferricyanide in the presence of Tocopherol only. This reaction is dependent upon prior dark incubation as though an oxidation-reduction equilibrium has to be reached before the reaction proceeds. With chloroplasts, after correction for proper controls, it is found that Tocopherol does not act catalytically but only in substrate amounts. An equivalence is found between ferricyanide reduced and Tocopherol oxidized. At the present time this reactivation is not clear.

Occasionally, but with sufficient frequency to be encouraging, we are able to completely reactivate inactive chloroplasts with a protein fraction prepared from "normal" active chloroplasts. Attempts are being made to improve conditions of isolation of this protein fraction so that we can routinely prepare this fraction and study some properties of it.

Oxygen Exchange in Chloroplasts

Illumination of chloroplasts (as normally prepared) results in an oxygen exchange. Oxygen is produced and taken up simultaneously. The rate of production of oxygen is approximately one-tenth that of a rapid Hill reaction. This exchange rate may be enhanced by addition of any of a number of autooxidizable compounds to the reaction mixture. The stoichiometry of 1:1 is observed in the presence of catalase. If a hydrogen peroxide trapping system is employed, the ratio now changes so that two oxygen molecules are taken up for every one evolved. A generally used trapping system is ethanol which is oxidized by H_2O_2 to give acetaldehyde. With the exception of indophenol dyes, acetaldehyde is found. The seemingly unique indophenol system, while exhibiting a stoichiometry of 2:1, does not yield acetaldehyde when ascorbate is present. In some manner the presence of ethanol or ethanol and catalase, results in a photooxidation of ascorbate by the chloroplasts. The mechanism of this oxidation is being pursued.

Photosynthetic Phosphorylation

A unique relationship exists between the ability of certain compounds to uncouple phosphorylation and their action as inhibitors of electron transport. Both ammonia and quinicrine uncouple phosphorylation from electron transport, thereby increasing the rate of the Hill reaction. If, however, the concentration of these two compounds is increased also oxygen evolution is inhibited. On the other hand, the reduction of pyridine nucleotide, when reduced indophenol dyes are used as electron donors, is not inhibited. The data suggest a more intricate relationship between photophosphorylation and photoevolution of oxygen than generally assumed. To test such a possible interaction between these systems we have attempted to reverse this inhibition by uncouplers by adding various compounds. No satisfactory restoration of oxygen evolution has been found. However, in the case of quinicrine, several interesting results were noted. The extent of inhibition was time-dependent in dark and occurs much more rapidly in the light. Interestingly in this system the presence of ATP has a small reversing effect on the rate of the Hill reaction and on the total amount of pyridine nucleotide formed in an inhibited system. No such effect has been found with ammonia.

Studies of Ochromonas

Work is progressing on a study of the Chrisomonad Ochromonas danica. This organism shows several features which make it a promising

source of information concerning photosynthesis. It shares with many other organisms a lack of chlorophyll b or bilin pigments as accessory sensitizers. Preliminary spectroscopic studies showed that chlorophyll a occurs in different binding states, one of which is located at 695 mu. This long wave chlorophyll observed both in whole cells and in cell free extracts, behaved in a quite peculiar fashion - its main feature being an extreme lability. A survey has been made of conditions which caused disappearance of this long wave chlorophyll. Since so far no satisfactory explanation for this long wave chlorophyll and its chemical interactions have been found, a discussion of these experiments will be deferred to a later date. We are presently expanding our studies to the various parameters of the photosynthetic activity of whole Ochromonas cells and of cell free extracts obtained thereof. Attempts are being made to obtain activation spectra of photosynthesis. The first question to be answered is whether or not there is a long wave drop of the quantum efficiency. Enhancement experiments then must follow to further decide whether or not in this organism there are two or only one photoreaction. Presently we suspect there is only one photoact, namely, one responsible for the evolution of oxygen.

Studies of a Scenedesmus Mutant

Another attempt to use an anomalous organism to elucidate the mechanism of photosynthesis concerns a study of a mutant of Scenedesmus.

This mutant, isolated by Dr. Norman Bishop, is unable to fix CO_2, and as far as we can tell, devoid of pigment P700. We have made spectroscopic studies of the mutant and investigated some of its fluorescence characteristics. After it proved possible to obtain active cell free preparations, a survey was made of the capacity of these particles for photoreducing the various materials. The result of this survey proved very interesting, mainly because it does not seem to fit our present concept of photosynthetic electron transport. One of the peculiarities we are presently trying to resolve is that particles prepared from this mutant can reduce low potential oxidants such as indigocarmin and viologen, but seem unable to carry out the reduction of TPN. Also, the correlation between the redox potential of the various oxidants used as a substrate and the quantum yields observed in their photoreduction fails to follow the expectations. At present we have no satisfactory hypothesis to explain these observations. This is the more intriguing since preliminary data concerning the activation spectrum of photosynthesis in this organism and also recent fluorescence measurements clearly indicate that photosystem I, as it is presently conceived, is fully inoperative. Drs. Beinert (Madison, Wisconsin) and Weaver (Stanford, California), are presently collaborating with us to answer the question whether or not this mutant shows a significant fast decaying ESR signal, generally assumed to accompany photosystem I.

Difference Spectroscopy

During the past few months we have been concentrating on attempts to improve the time resolution of our difference spectroscopy. A number of improvements of technical nature have yielded a resolution which is compatible with that of the CAT (computer of average transients) which is equivalent to 60 usec per station. While still in the developmental stage, the apparatus was able to observe in chloroplasts a very fast decaying photoconversion and possibly a consecutive reaction with a half-time of 100-200 usec. The two species involved in all likelihood, are P700 and an unknown reaction partner, absorbing at 420 mu. Presently we are trying to improve performance of this apparatus in the red spectral region and further biochemical studies will be deferred until some remaining technical difficulties are solved.